Use of Mathematical Models to Estimate Characteristics of Pyrethroid Resistance in Tobacco Budworm and Bollworm (Lepidoptera: Noctuidae) Field Populations

M. J. LIVINGSTON,[1] G. A. CARLSON,[2] AND P. L. FACKLER[2]

J. Econ. Entomol. 95(5): 1008–1017 (2002)

ABSTRACT Genetic models have been used to examine the evolution of insecticide resistance in pest species subject to data and assumptions regarding genetic, biological, and operational parameters. We used time-series data on pyrethroid tolerance and simple genetic models to estimate underlying genetic and biological parameters associated with resistance evolution in tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie), Louisiana field populations. Assuming pyrethroid resistance is conferred by one gene at one locus in both species, inheritance of pyrethroid resistance was partially dominant in the tobacco budworm and partially recessive in the bollworm. Relative fitness estimates indicated that fitness costs associated with resistance selected against resistance alleles in the absence of selection pressure in the tobacco budworm, but not in the bollworm. In addition, relative fitness estimates obtained using the indirect method outlined in this study were similar in magnitude to estimates obtained using traditional direct approaches.

KEY WORDS *Heliothis virescens*, *Helicoverpa zea*, pyrethroid resistance, mathematical model, relative fitness estimates, fitness cost

THE DEVELOPMENT OF organochlorine, organophosphate, and carbamate resistance in the tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) (Sparks 1981, Wolfenbarger et al. 1981), its status as a major cotton pest, *Gossypium hirsutum* (L.) (Luttrell 1994), and reports of increased pyrethroid tolerance in 1986 in Arkansas, Louisiana, Mississippi, and Texas (Plapp et al. 1987, Roush and Luttrell 1987, Leonard et al. 1988, Plapp et al. 1990b) stimulated over a decade's worth of research on the nature of pyrethroid resistance in this species. Resistance is now widespread in midsouth populations and has eliminated pyrethroids as an effective control (Bagwell et al. 2000). Resistance has been associated with nerve insensitivity (Taylor et al. 1993, Taylor et al. 1996, Lee et al. 1999), enhanced metabolism (Nicholson and Miller 1985, Plapp et al. 1990a), and reduced penetration and frequencies of larvae and adults expressing nerve insensitivity and enhanced metabolism have been shown to fluctuate during the growing season (Ottea et al. 1995, Ottea and Holloway 1998). Inheritance has been characterized as recessive (Plapp et al. 1990a), partially recessive ($0 < d < 0.5$, Payne et al. 1988, Watson and Kelly 1991), and partially dominant ($0.5 < d < 1.0$, Elzen et al. 1994), where d denotes the degree of dominance constrained to the unit interval (Bourget et al. 2000). Resistance instability in the absence of selection pressure has been reported (Nicholson and Miller 1985, Staetz 1985, Leonard et al. 1987, Payne et al. 1988, Campanhola and Plapp 1989, Elzen et al. 1994, Ottea et al. 1995), as have potential fitness costs associated with resistance. Campanhola et al. (1991) reported that laboratory-selected resistant females were less able to produce viable offspring than susceptible females, and that resistant females produced less pheromone and were less attractive to males than susceptible females. Fecundity differentials between resistant and susceptible females were density dependent, being largest at the beginning of the cotton-growing season when populations were low. Reduced nerve sensitivity has been related to at least two distinct genetic mutations (Park and Taylor 1997, Park et al. 1997, Park et al. 2000), both of which adversely affected sodium-channel gating properties of central neurons in the absence of selection pressure (Lee et al. 1999, Zhao et al. 2000). One of the alleles was associated with a smaller fitness cost and, as a result, may have succeeded the allele with the larger fitness cost in Louisiana populations during the 1990s (Park 1998, Zhao et al. 2000).

The bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), another major cotton pest (Luttrell 1994), has also developed organochlorine and organophosphate resistance (Sparks 1981, Wolfenbarger et al. 1981), but has only recently exhibited increased pyrethroid tolerance (Abd-Elghafar et al. 1993, Kanga

[1]United States Department of Agriculture, Animal and Plant Health Inspection Service, Policy and Program Development, Policy Analysis and Development, 4700 River Road, Unit 119, Riverdale, MD 20737 (e-mail: michael.j.livingston@aphis.usda.gov).

[2]Department of Agricultural and Resource Economics, North Carolina State University, Box 8109, Raleigh, NC 27695.

0022-0493/02/1008–1017$02.00/0

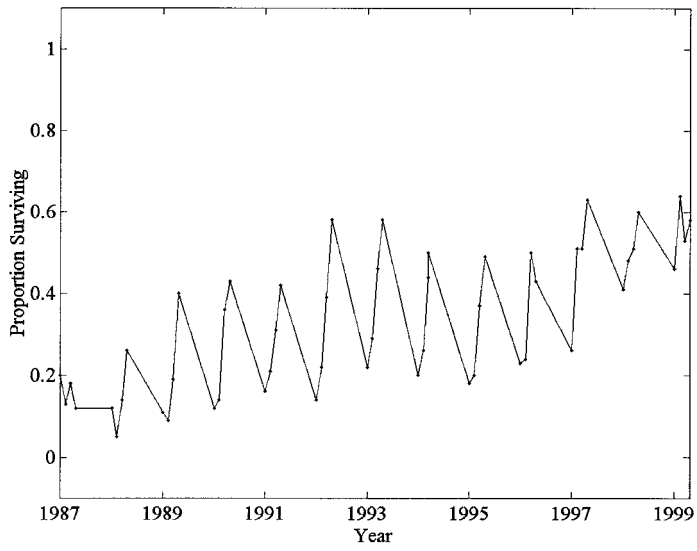

Fig. 1. Monthly average survival rates of adult male tobacco budworms subjected to a diagnostic dose of 10 μg cypermethrin per vial. May, June, July, and August survival rates were averaged over Louisiana cotton production regions during the 1987 through 1999 growing seasons (Bagwell et al. 2000). Hash marks indicate the first observation for the given year.

et al. 1996, Brown et al. 1998, Bagwell et al. 2000). Pyrethroid resistance is apparently a localized phenomenon in this species (Kanga et al. 1996, Brown et al. 1998), and pyrethroid insecticides remain an effective bollworm control throughout the midsouth (Bagwell et al. 2000). Resistance has been associated with nerve insensitivity (Ottea and Holloway 1998), enhanced metabolism (Abd-Elghafar et al. 1993, Kanga et al. 1996), and reduced penetration (Abd-Elghafar and Knowles 1996). Lambda-cyhalothrin LD_{50} values for South Carolina adults in vial tests indicated that inheritance was partially dominant in 1996 (Brown et al. 1998). Kanga et al. (1996) reported that resistance was unstable in the absence of selection pressure, citing fitness costs associated with resistance as a potential factor.

A voluntary resistance management strategy for the midsouth was initiated in the late 1980s that restricted pyrethroid use to the midseason (Plapp et al. 1990a, Elzen et al. 1992). Adult vial test data (Bagwell et al. 2000) indicate that pyrethroid tolerance levels in Louisiana tobacco budworms were lowest in May, increased dramatically in late spring and summer during the window of increased pyrethroid use, and declined sharply the following May during each of the 1987 through 1999 growing seasons (Fig. 1). A similar pattern was observed for this species in vial test data reported in Arkansas, MS, and Texas from 1986 through 1988 (Plapp et al. 1990b). Vial test data indicate a somewhat similar, though much less regular and dramatic pattern for Louisiana bollworm populations during the 1988 through 1999 growing seasons (Fig. 2).

Several factors may have contributed to the patterns exhibited in the vial test data. First, fitness costs associated with resistance may have selected against resistance alleles in the absence of selection pressure. Second, the susceptibility of adults, regardless of genotype, to vial tests may have depended on when the bioassays were performed. At the beginning of the year, for example, adults may have been less able to survive the bioassay than later in the year, having developed on plant hosts of relatively poor quality. That is, patterns observed for pyrethroid tolerance may, in part, be due to environmental effects on fitness that do not depend on resistance. Third, since both species are highly mobile (Rabb and Kennedy 1979, Kennedy and Storer 2000), migration may have played a role (e.g., Roush and McKenzie 1987). Immigration of susceptible adults has been linked to pyrethroid resistance reversion in Australian *Helicoverpa armigera* (Hübner) populations (Daly and Fitt 1990, Forrester et al. 1993). Immigration of resistant adults, however, can also lead to resistance evolution (Caprio and Tabashnik 1992, Croft and Dunley 1993). Sorenson et al. (1998) reported increased pyrethroid tolerance in Missouri bollworms in regions where no pyrethroids were used, citing immigration of resistant moths as the causal factor.

The objective of this study was to determine if simple genetic models can be used to estimate genetic and biological parameters involved in historic patterns of resistance evolution (Figs. 1 and 2). Taylor et al. (1983) tested the ability of single-locus genetic models to predict dieldrin resistance allele frequencies in laboratory populations of the house fly, *Musca domestica* (L.), in five different selection experiments. The sum of squared deviations between observed and predicted resistance allele frequencies after four generations was low, indicating that their simple models characterized resistance evolution well in a laboratory environment. Tabashnik and Croft (1985) used genetic models successfully to predict resistance onset in

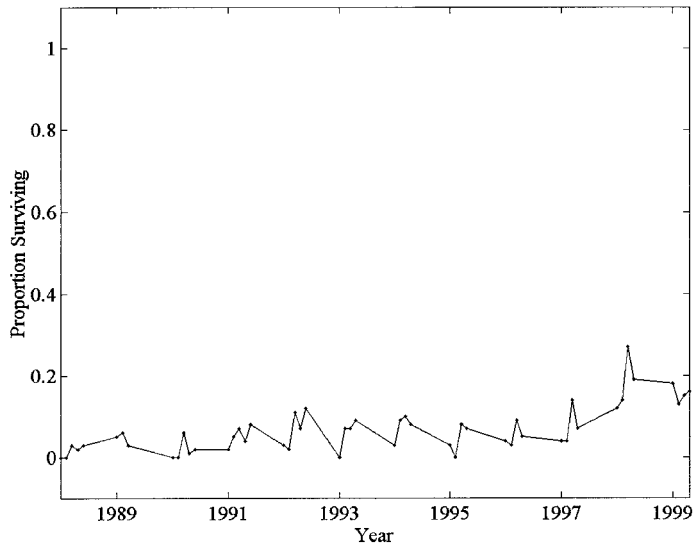

Fig. 2. Monthly average survival rates of adult male bollworms subjected to 5 μg cypermethrin per vial. Various monthly survival rates were averaged over Louisiana cotton production regions during the 1988 through 1999 growing seasons (Bagwell et al. 2000). Hash marks indicate the first observation for the given year.

12 species of apple crop pests and 12 species of natural enemies using field data. Typically, genetic models have been used to simulate the evolution of insecticide resistance subject to data and assumptions regarding model parameters. In this study, we used time-series data on pyrethroid tolerance (Figs. 1 and 2) and simple genetic models with no migration to estimate genetic and biological parameters associated with resistance evolution in tobacco budworm and bollworm Louisiana field populations. We ignore the impact of migration on pyrethroid tolerance because its incorporation was not possible due to data limitations.

Materials and Methods

Data. During the 1987–1999 growing seasons, Louisiana researchers collected adult male tobacco budworms and bollworms in pheromone traps placed throughout Louisiana's cotton-growing regions (Bagwell et al. 2000). Budworms and bollworms were subjected to 10 and five micrograms of cypermethrin, respectively, in vial test bioassays described by Plapp et al. (1987). A diagnostic dose was used for the tobacco budworm but not for the bollworm. Fig. 1 shows May through August statewide average survival rates for the tobacco budworm, which provide estimates of the proportions of males homozygous for pyrethroid resistance. For the bollworm, average survival rates were reported for May through September in 1988, 1990, 1991, and 1992; for July through September in 1989; for June through September in 1993; and for May through August in 1994 through 1999 (Fig. 2). Both figures reveal interseasonal patterns of tolerance reversion and intraseasonal patterns of increased tolerance, although these patterns are much more regular and dramatic for the tobacco budworm.

Simulation model. A two-locus, four-allele, deterministic model was used to simulate the evolution of pyrethroid and transgenic insecticidal cotton (Bt) resistance in both species. The evolution of Bt resistance was modeled because Bt cotton has been widely adopted in Louisiana since its commercial availability in 1996 (Williams 1997, 1998, 1999, 2000), and because Bt cotton and pyrethroids have been used in concert to control bollworm populations (Sims 1995, Layton et al. 1997, Carter 1998, Lambert et al. 1998), leading to potential toxin-mixture effects on pyrethroid resistance evolution in both species (Georghiou 1983, Curtis 1985, Gould 1986, Mani 1985, Taylor 1986, Caprio 1998). In particular, unpublished simulations conducted by the authors have suggested that grower use of Bt cotton in the midsouth may lead to reductions over time in pyrethroid resistance allele frequencies in the tobacco budworm. As a result, we believe that parameters that characterize pyrethroid resistance evolution should be estimated using a simulation model that also incorporates Bt resistance evolution.

For simplicity, population dynamics, migration, and interseasonal fitness costs associated with pyrethroid resistance, such as reduced winter survival of resistant genotypes, were not modeled. Selection occurred under the following seven assumptions: (1) random mating, diploid with no sex linkage; (2) one-to-one sex ratio; (3) mutation was insignificant relative to selection as a mechanism of resistance evolution over the sample period; (4) linkage equilibrium; (5) larval selection; (6) migration did not affect resistance evolution; and (7) resistance to each toxin was conferred at one locus by one allele. Changes in resistance allele frequencies were allowed to occur at each time step, which represented one generation. There were four gametes and nine genotypes (Table 1). Let pyrethroid

Table 1. Genotypes, frequencies of genotypes in the progeny of generation *i* adults during growing season *t*, and genotype rates of surviving and reproducing successfully under Bt, pyrethroid, and no selection pressure

Genotypes	Frequencies ($f_{t,i+1}$)	Bt fitnesses (s^b)	Pyrethroid fitnesses (s^p)	No Bt fitnesses (s^{ab})	No pyrethroid fitnesses (s^{ap})
xy/xy	$g^1_{t,i}{}^2$	RR^{Bt}	RR^p	RR^{aBt}	RR^{ap}
xy/xY	$2\ g^1_{t,i}g^2_{t,i}$	RS^{Bt}	RR^p	RS^{aBt}	RR^{ap}
xY/xY	$g^2_{t,i}{}^2$	SS^{Bt}	RR^p	SS^{aBt}	RR^{ap}
xy/Xy	$2\ g^1_{t,i}g^3_{t,i}$	RR^{Bt}	RS^p	RR^{aBt}	RS^{ap}
xy/XY-xY/Xy	$2(g^1_{t,i}g^4_{t,i} + g^2_{t,i}g^3_{t,i})$	RS^{Bt}	RS^p	RS^{aBt}	RS^{ap}
xY/XY	$2\ g^2_{t,i}g^4_{t,i}$	SS^{Bt}	RS^p	SS^{aBt}	RS^{ap}
Xy/Xy	$g^3_{t,i}{}^2$	RR^{Bt}	SS^p	RR^{aBt}	SS^{ap}
Xy/XY	$2\ g^3_{t,i}g^4_{t,i}$	RS^{Bt}	SS^p	RS^{aBt}	SS^{ap}
XY/XY	$g^4_{t,i}{}^2$	SS^{Bt}	SS^p	SS^{aBt}	SS^{ap}

resistance and susceptibility be conferred to individuals at locus one by alleles *x* and *X*, let Bt resistance and susceptibility be conferred at locus two by alleles *y* and *Y*, and let $x_{t,i}$, $X_{t,i}$, $y_{t,i}$, and $Y_{t,i}$ denote allele frequencies for generation *i* adults during growing season *t*. Under assumption (iv), gamete frequencies were products of the frequencies of the alleles making up the gametes

$$g^1_{t,i} = x_{t,i}y_{t,i},\ g^2_{t,i} = x_{t,i}Y_{t,i},\ g^3_{t,i} = X_{t,i}y_{t,i},\ g^4_{t,i} = X_{t,i}Y_{t,i}. \quad [1]$$

Larval genotype frequencies ($f_{t,i+1}$) were then functions of the gamete frequencies (Table 1).

We assumed five discrete generations per year for both species (Livingston 1999). In addition, we assumed that larvae faced four different selection environments. Table 2 reports the default proportions of each species in cotton and the default proportions in cotton treated with pyrethroids for each generation. Proportions of either species not in cotton were assumed to be in wild hosts or noncotton cultivated hosts that were not treated with pyrethroids. Let b_t denote the proportion of Bt cotton planted in Louisiana during growing season *t*, let c_{i+1} denote the proportion in cotton at the beginning of generation $i+1$, and let s_{i+1} denote the proportion in cotton selected for pyrethroid resistance. Then the selection environments and the proportions of larvae facing each were: selection for Bt resistance, $b_tc_{i+1}(1\text{-}s_{i+1})$; selection for Bt and pyrethroid resistance, $b_tc_{i+1}s_{i+1}$; selection for pyrethroid resistance, $(1\text{-}b_t)c_{i+1}s_{i+1}$; and not selected, $ns_{t,i+1}$, the remainder.

The average fitness of larvae in generation $i+1$ was

$$w_{t,i+1} \equiv ns_{t,i+1} \cdot f_{t,i+1}{}' \cdot (s^{ab} \cdot *s^{ap}) + b_t \cdot c_{i+1} \cdot f_{t,i+1}{}' \cdot (s^b \cdot *s^{ap}) + b_t \cdot c_{i+1} \cdot s_{i+1} \cdot f_{t,i+1}{}' \cdot s^{bp} + (1 - b_t) \cdot c_{i+1} \cdot s_{i+1} \cdot f_{t,i+1}{}' \cdot (s^p \cdot *s^{ab}) \quad [2]$$

Table 2. Parameters, sources, and ranges for Bt-resistance parameters explored in the sensitivity analysis

Parameter	Pyrethroids	Population proportions	Bt
	Tobacco budworm		
Initial R-allele frequency	0.4472 (Bagwell et al. 2000)	—	1.5e-3 (Gould et al. 1997)
Treated fitness homozygote	0.712 (Elzen et al. 1994)	—	0.950 (No data)
Treated fitness heterozygote	0.4095 (Elzen et al. 1994)	—	0.002 (Suszkiw 2001, see *Materials and Methods*)
Treated fitness susceptible	0.033 (Elzen et al. 1994)	—	0.001 (Livingston 1999)
Untreated fitness homozygote	— (No data)	—	0.950 (No data)
Untreated fitness heterozygote	— (No data)	—	0.995 (No data)
Untreated fitness susceptible	1.000 (No data)	—	1.000 (No data)
Proportion in cotton, May–August	—	0.980 (No data)	—
	Bollworm		
Initial R-allele frequency	— (No data)	—	1.0e-4 (Burd et al. 2001)
Treated fitness homozygote	— (No data)	—	0.950 (No data)
Treated fitness heterozygote	— (No data)	—	0.775 (Burd et al. 2000)
Treated fitness susceptible	0.027 (Sparks 1981, Elzen et al. 1994)	—	0.250 (Storer 1999)
Untreated fitness homozygote	— (No data)	—	0.950 (No data)
Untreated fitness heterozygote	— (No data)	—	0.9625 (No data)
Untreated fitness susceptible	1.000 (No data)	—	1.000 (No data)
Proportion in cotton, May and June	—	0.100 (No data)	—
Proportion in cotton, July and Aug.	—	0.800 (No data)	—
	Both species		
Proportion in cotton, April	—	0.050 (No data)	—
Proportion sprayed, April–June	—	0.000 (No data)	—
Proportion sprayed, July	—	0.750 (No data)	—
Proportion sprayed, August	—	0.375 (No data)	—

where $'$ denotes the transpose operator, and * denotes element-by-element multiplication. The proportion of pyrethroid resistance alleles contributed to the adult population by surviving larvae was

$$m_{t,i+1} \equiv ns_{t,i+1} \cdot (s^{ab}(1{:}6) \cdot {*} s^{ap}(1{:}6)) + b_t \cdot c_{i+1} \cdot (s^{b}(1{:}6) \cdot {*} s^{ap}(1{:}6)) + b_t \cdot c_{i+1} \cdot s_{i+1} \cdot s^{bp}(1{:}6) + (1 - b_t) \cdot c_{i+1} \cdot s_{i+1} \cdot (s^{p}(1{:}6) \cdot {*} s^{ab}(1{:}6)), \quad [3]$$

where (1:6) denotes vector elements one through six, with the first three elements of $m_{t,i+1}$ postmultiplied by 2. The proportion of Bt resistance alleles contributed to the adult population by surviving larvae was

$$n_{t,i+1} \equiv ns_{t,i+1} \cdot (s^{ab}(\iota) \cdot {*} s^{ap}(\iota)) + b_t \cdot c_{i+1} (s^{b}(\iota) \cdot {*} s^{ap}(\iota)) + b_\iota \cdot c_{i+1} \cdot s_{i+1} \cdot s^{bp}(\iota) + (1 - b_\iota) \cdot c_{i+1} \cdot s_{i+1} \cdot (s^{p}(\iota) \cdot {*} s^{ab}(\iota)), \quad [4]$$

where (ι) denotes vector elements 1, 2, 4, 5, 7, and 8, with elements 1, 3, and 5 of $n_{t,i+1}$ postmultiplied by 2. Pyrethroid and Bt resistance allele frequencies in the adult population were then

$$x_{t,i+1} \equiv \frac{f_{t,i+1}{}'(1{:}6) \cdot m_{t,i+1}}{2w_{t,i+1}} \qquad y_{t,i+1} \equiv \frac{f_{t,i+1}{}'(\iota) \cdot n_{t,i+1}}{2w_{t,i+1}}. \quad [5]$$

Equations 1 through 5 were used to simulate the intraseasonal resistance evolution dynamics. To simulate the interseasonal dynamics, resistance allele frequencies for the first generation of adults in the following growing season were

$$x_{t+1,1} \equiv \frac{f_{t,5+1}{}'(1{:}6) \cdot m_{t,5+1}}{2w_{t,5+1}} \qquad y_{t+1,1} \equiv \frac{f_{t,5+1}{}'(\iota) \cdot n_{t,5+1}}{2w_{t,5+1}}. \quad [6]$$

Bt-Resistance Parameters. We based Bt-resistance parameters on available laboratory studies, because field data on Bt tolerance were unavailable (Table 2). We used a different method, however, to set the relative fitness of budworms heterozygous for Bt resistance. We did this for three reasons. First, available laboratory estimates were not consistent with the fact that changes in tolerance to Bt cotton have not been observed in the tobacco budworm by the Bt resistance-monitoring team responsible for reporting changes in susceptibility (Suszkiw 2001). Second, laboratory estimates may not be appropriate for characterizing resistance evolution under field conditions (Bourget et al. 2000). Third, this parameter is a critical determinant of the rate of Bt resistance evolution. Therefore, we used a single-gene version of the genetic model and historic data on Bt cotton use in Louisiana and Mississippi to find the highest level of heterozygous fitness consistent with no change in Bt tolerance and used this as the default level (Table 2).

Estimation Problems. Assumptions 1 through 7, equations 1 through 6, and Tables 1 and 2 specify the genetic model and known parameters. For the tobacco budworm, we minimized the sum of squared deviations (SSD) between the genetic model's predictions concerning the proportions of individuals homozygous for pyrethroid resistance, $e_i \cdot x_{t,i+1}\ (\Lambda)$, and the actual proportions of resistant homozygotes (Bagwell et al. 2000), $x_{t,i+1}{}^2$, with respect to the pyrethroid-resistance (Λ) and environmental fitness ($0 \leq e_i \leq 1$) parameters,

$$\mathrm{SSD} = \sum_{t=1}^{T} \sum_{i=2}^{5} (x_{t,i+1}{}^2 - e_i \cdot x_{t,i+1}{}^2(\Lambda))^2 \quad [7]$$

where T denotes the number of years of vial test data. Environmental fitness parameters were constrained to the unit interval; values less than one indicated the presence of a biological impediment to fitness independent of resistance, and values equal to one indicated no biological impediment to fitness. Environmental fitness parameters entered the tobacco budworm's model by potentially reducing proportions of homozygotes able to survive the vial test bioassay.

For the bollworm, we minimized the sum of squared deviations between the genetic model's predictions concerning fitness in the vial test, $e_i \cdot w^p{}_{t,i}\ (\Lambda)$, and the actual fitnesses (Bagwell et al. 2000), $w^p{}_{t,i}$, with respect to the pyrethroid-resistance and environmental fitness parameters,

$$\mathrm{SSD} = \sum_{t=1}^{T} \sum_{i=f(t)}^{l(t)} (w^p{}_{t,i} - e_i \cdot w^p{}_{t,i}(\Lambda))^2 \quad [8]$$

where

$$w^p{}_{t,i}(\Lambda) \equiv f_{t,i+1}{}' \cdot s^p. \quad [9]$$

The five discrete generations assumed to occur for both species were assumed to coincide in length with the months April, May, June, July, and August, so that the predictions of the genetic models could be matched up with the monthly vial test data. In addition, we assumed that adult survival rates were perfectly correlated with larval survival rates (Gage and Hatfield 1989, Roush and Luttrell 1989, Plapp et al. 1990a, Plapp et al. 1990b).

Several features of the least-squares estimation problems 7 and 8 deserve comment. First, because parameters entered the genetic models nonlinearly, closed-form solutions did not exist. As a result, an iterative procedure was needed to obtain estimates. Second, all parameter estimates were constrained to the unit interval. We used a constrained Levenberg-Marquardt procedure with line search because it is robust and efficient in this case (Fletcher 1987, Gallant 1987, Greene 1993). In particular, we solved each minimization problem using the *lsqnonlin* function in Matlab (Coleman et al. 1999). Third, data on pyrethroid resistance allele frequencies were not used to predict resistance allele frequencies in subsequent generations. Resistance allele frequencies were always simulated using the genetic model. Fourth, iter-

Table 3. Least-squares biological parameter estimates

Parameter	Unrestricted estimate (±SE)	95% CI	Restricted estimate (± SE)	95% CI
	Tobacco budworm model[a]			
Initial allele frequency ($x_{1987,2}$)	0.4428 (0.0697)****	[0.30, 0.58]	fixed at 0.4472	—
Treated fitness homozygote (RR^p)	0.6506 (0.5879)	[0.00, 1.00]	fixed at 0.7120	—
Treated fitness heterozygote (RS^p)	0.4845 (0.7577)	[0.00, 1.00]	fixed at 0.4095	—
Treated fitness susceptible (SS^p)	fixed at 0.0330	—	fixed at 0.0330	—
Untreated fitness homozygote (RR^{ap})	0.8164 (0.1442)****	[0.53, 1.00]	0.7908 (0.0082)****	[0.77, 0.81]
Untreated fitness heterozygote (RS^{ap})	0.8625 (0.2513)**	[0.36, 1.00]	0.8861 (0.0214)****	[0.84, 0.93]
Untreated fitness susceptible (SS^{ap})	fixed at 1.0000	—	fixed at 1.0000	
May environmental factor (e_2)	0.5210 (0.0576)****	[0.41, 0.64]	0.5146 (0.0527)****	[0.41, 0.62]
June environmental factor (e_3)	0.7154 (0.0624)****	[0.59, 0.84]	0.7192 (0.0599)****	[0.60, 0.84]
	Bollworm model[b]			
Initial allele frequency ($x_{1988,2}$)	0.0505 (0.3262)	[0.00, 0.71]	0.0510 (0.1031)	[0.00, 0.26]
Treated fitness homozygote (RR^p)	0.5826 (1.5800)	[0.00, 1.00]	0.5734 (0.1384)***	[0.29, 0.85]
Treated fitness heterozygote (RS^p)	0.1349 (0.8010)	[0.00, 1.00]	0.1324 (0.1836)	[0.00, 0.50]
Treated fitness susceptible (SS^p)	fixed at 0.0270	—	fixed at 0.0270	—
Untreated fitness homozygote (RR^{ap})	1.0000 (0.5037)*	[0.00, 1.00]	fixed at 1.0000	—
Untreated fitness heterozygote (RS^{ap})	0.9986 (0.1691)****	[0.66, 1.00]	fixed at 1.0000	—
Untreated fitness susceptible (SS^{ap})	fixed at 1.0000	—	fixed at 1.0000	—
May environmental factor (e_2)	0.4728 (0.1057)***	[0.26, 0.69]	0.4732 (0.1006)****	[0.27, 0.68]
June environmental factor (e_3)	0.4103 (0.0875)****	[0.23, 0.59]	0.4104 (0.0844)****	[0.24, 0.58]

*, Statistically different from zero at the 0.0001****, 0.001***, 0.01**, and 0.1* significance levels.
[a] Unrestricted: $\bar{R}^2 = 0.76$, SEE = 0.0068, $n = 52$, df = 45. Restricted: $\bar{R}^2 = 0.78$, SEE = 0.0064, $n = 52$, df = 48.
[b] Unrestricted: $\bar{R}^2 = 0.58$, SEE = 0.3346, $n = 45$, df = 38. Restricted: $\bar{R}^2 = 0.60$, SEE = 0.3179, $n = 45$, df = 40.

ative procedures do not guarantee the acquisition of global solutions to nonlinear least-squares estimation problems. Initial starting values for each estimated parameter and numerical criteria for terminating the procedure were required. When several local minima exist, parameter estimates can vary with these specifications. We terminated every minimization procedure when the sum of squared deviations and associated parameter estimates changed by <1e-7 in successive iterations. Initial starting values were based on published estimates when these were available. When initial starting values were not available for a particular parameter, we solved the estimation problem using several different initial values. In all cases, parameter estimates were insensitive to initial conditions. Estimates providing the highest coefficients of determination were reported.

Fifth, we solved unrestricted and restricted estimation problems. Initially, all of the parameters defined above, including initial pyrethroid resistance allele frequencies, were estimated in preliminary estimation problems. Relative fitness parameter estimates, however, were highly covariant and could not be estimated precisely simultaneously. Therefore, we fixed fitnesses for susceptible genotypes at published estimates (Table 2) (under selection pressure) and at unity (in the absence of selection pressure). In addition, environmental fitnesses not statistically different from unity in preliminary estimations were fixed at unity in the final unrestricted estimation results reported in Table 3. By fixing parameters at published values (Table 2) or unity and solving restricted estimation problems, we were able to improve the precision of the remaining parameter estimates. Sixth, few studies have examined the nature of pyrethroid resistance in the bollworm, perhaps because resistance has been slow to develop in this species. As a result, few published parameter estimates were available to specify the model for this species and to assess the consistency of our estimates. Sparks (1981) reported ratios of budworm-to-bollworm pyrethroid LD_{50} or LC_{50} values that had been reported in studies published shortly before and shortly after pyrethroids were commercially available. The average of the ratios reported by Sparks (1981), weighted by the number of studies used to calculate each ratio, was 1.21. We divided the estimate of the relative fitness of susceptible tobacco budworms under selection pressure, 0.033, by 1.21 and used this, 0.027, as an estimate of the relative fitness of susceptible bollworms under selection pressure (Table 2). Seventh, parameter estimates varied with the proportions of both species in cotton during the growing season. Because default specifications for these parameters were based on subjective assessments, we examined relationships between these specifications and the parameter estimates in a sensitivity analysis.

Results

Tobacco Budworm. Parameter estimates for the tobacco budworm are reported in Table 3. The unrestricted and restricted models explained 79% of the variation in the vial test data, and the adjusted coefficient of determination was slightly higher in the restricted model (0.78) than it was in the unrestricted model (0.76). Unrestricted relative fitness estimates under selection pressure were not statistically different from zero because both parameters were covariant, $\text{Cov}(RR^p, RS^p) = 0.4224$; however, the levels of the estimates 0.65 and 0.49 (Table 3) were similar to the published estimates 0.71 and 0.41 (Table 2). Pub-

Table 4. Sensitivity of unrestricted least-squares estimates in the tobacco budworm model to the proportion of the population in cotton May–August

	Proportion in cotton					
	0.75	0.80	0.85	0.90	0.95	0.98
Standard error of the estimate	0.0071	0.0071	0.0070	0.0069	0.0069	0.0068
Initial allele frequency ($x_{1987,2}$)	0.4537	0.4577	0.4607	0.4579	0.4487	0.4428
Treated fitness homozygote (RR^p)	1.0000	1.0000	0.9936	0.8905	0.7232	0.6506
Treated fitness heterozygote (RS^p)	0.9762	0.9548	0.9250	0.7834	0.5723	0.4845
Untreated fitness homozygote (RR^{ap})	0.8444	0.8240	0.8041	0.8003	0.8123	0.8164
Untreated fitness heterozygote (RS^{ap})	0.8251	0.8090	0.7951	0.8060	0.8434	0.8625
May environmental factor (e_2)	0.5252	0.5226	0.5199	0.5190	0.5204	0.5210
June environmental factor (e_3)	0.7041	0.7051	0.7064	0.7087	0.7125	0.7154

lished estimates were given by genotype survival rates in spray chamber bioassays (Elzen et al. 1994); however, fitness in our model is defined as the rate a genotype survives exposure to pyrethroids in the field and successfully reproduces. The result that our fitness estimates differed slightly from those reported by Elzen et al. (1994), therefore, is reasonable. Standard errors for the remaining estimates were considerably lower, and the unrestricted estimate of the initial resistance allele frequency (0.44) was very similar to the published estimate (0.45).

The precision of the estimates was improved considerably in the restricted model, because the number of parameters estimated was reduced and because more information was incorporated in the simulation model. Restricted relative fitness estimates in the absence of selection pressure were 0.79 for the resistant homozygote and 0.89 for the heterozygote and were statistically different from zero and unity at the 5% significance level. These estimates were similar to relative fitness estimates reported for the saw-toothed grain beetle, *Oryzaephilu surinamensis* (L.) (Coleoptera: Silvanidae), in the absence of malathion selection pressure (Muggleton 1986, Mason 1998). Muggleton (1986) reported relative fitness estimates for resistant homozygotes and heterozygotes of 0.82 for both genotypes, assuming resistance was conferred by one dominant allele at one locus. Mason (1998) reported relative fitness estimates of 0.92, 0.83, and 0.68 for both genotypes in three experiments under the same assumption. Finally, environmental effects on fitness in May and June were statistically different from unity at the 5% significance level in the unrestricted and restricted models, suggesting that tobacco budworms may have been less able to survive the vial test bioassay at the beginning relative to the end of the growing season. Because migration and interseasonal fitness costs were not incorporated in the simulation model, however, this finding should be interpreted with caution.

Bollworm. Parameter estimates for the bollworm are also reported in Table 3. The unrestricted and restricted models explained 64% of the variation in the vial test data, and the adjusted coefficient of determination was higher in the restricted model (0.60) than it was in the unrestricted model (0.58). Unrestricted estimates of the initial pyrethroid resistance allele frequency, relative fitnesses under selection pressure, and the relative fitness of the resistant homozygote in the absence of selection pressure were not statistically different from zero at the 5% significance level, due to parameter covariances: $\mathrm{Cov}(x_{1988,1},RR^p) = -0.2478$; $\mathrm{Cov}(x_{1988,1},RS^p) = -0.2601$; $\mathrm{Cov}(RR^p,RS^p) = 0.5874$; $\mathrm{Cov}(RR^p,RR^{ap}) = -0.5668$; $\mathrm{Cov}(RR^p,RS^{ap}) = -0.1481$; $\mathrm{Cov}(RS^p,RR^{ap}) = 0.1093$; and $\mathrm{Cov}(RS^p,RS^{ap}) = -0.1312$. Remaining unrestricted estimates were statistically different from zero at the 5% significance level, and the unrestricted estimate of the relative fitness of the resistant homozygote in the absence of selection pressure was significantly different from zero at the 10% level. Unrestricted relative fitness estimates for the resistant homozygote and heterozygote in the absence of selection pressure were close to unity, and the hypothesis that the latter was different from unity could not be rejected at the 5% significance level. Relative fitnesses for both genotypes in the absence of selection pressure, therefore, were fixed at unity in the restricted estimation problem to reduce the number of estimated parameters.

As was the case for the tobacco budworm, the precision of the parameter estimates was improved considerably in the restricted model. In particular, the restricted estimate of the relative fitness of the resistant homozygote under selection pressure was statistically different from zero at the 0.1% significance level. In addition, the 95% confidence intervals for initial resistance allele frequency and heterozygote relative fitness were much narrower in the restricted estimation problem. The degree of dominance implied by the restricted relative fitness estimates ($d = 0.19$) was much lower than the degree of dominance implied by LD_{50} values ($d = 0.63$) reported by Brown et al. (1998) for a population in South Carolina; however, because the 95% confidence intervals for the relative fitness estimates overlapped, the difference was not statistically significant. Finally, environmental effects on fitness in May and June were statistically different from unity at the 5% significance level in the unrestricted and restricted models, suggesting that bollworms may have been less able to survive the vial test bioassay at the beginning relative to the end of the growing season. Again, this finding should be interpreted with caution.

Table 5. Sensitivity of unrestricted least-squares estimates in the bollworm model to the proportion of the population in cotton May and June, and July and August

	Proportion in cotton					
	0.05, 0.75	0.10, 0.80	0.15, 0.85	0.20, 0.90	0.25, 0.95	0.30, 0.98
Standard error of the estimate	0.3341	0.3346	0.3352	0.3357	0.3363	0.3366
Initial allele frequency ($x_{1988,2}$)	0.0473	0.0505	0.0533	0.0564	0.0597	0.0614
Treated fitness homozygote (RR^p)	0.6698	0.5826	0.5193	0.4615	0.4095	0.3823
Treated fitness heterozygote (RS^p)	0.1431	0.1349	0.1306	0.1261	0.1218	0.1202
Untreated fitness homozygote (RR^{ap})	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Untreated fitness heterozygote (RS^{ap})	1.0000	0.9986	0.9947	0.9905	0.9855	0.9814
May environmental factor (e_2)	0.4722	0.4728	0.4730	0.4733	0.4739	0.4742
June environmental factor (e_3)	0.4096	0.4103	0.4110	0.4116	0.4125	0.4129

Sensitivity Analysis. Parameter estimates and standard errors of the estimates for different proportions of both species in cotton, including the default specifications, are reported in Tables 4 and 5. Parameter ranges were not based on data. For the tobacco budworm, relative fitnesses under selection pressure declined with the proportion in cotton, because the proportion selected for pyrethroid resistance increased with this parameter. For the bollworm, the relative fitness of the resistant homozygote under selection pressure declined with the proportion in cotton for the same reason. Remaining estimates were relatively insensitive to changes in the population proportions in cotton. For the tobacco budworm, the standard error of the estimate was lowest under the default specification. However, the standard error of the estimate for the bollworm was lowest when five and 75% of the population was in cotton during the early and late growing season, respectively, indicating that proportions in cotton may be lower than those assumed in the default model.

Discussion

The simple genetic model used in this study explained a significant amount of the variation in the vial test data. In addition, unrestricted parameter estimates for the tobacco budworm were very similar to previous estimates (Elzen et al. 1994, Bagwell et al. 2000), and relative fitness estimates in the absence of selection pressure were consistent with previous studies that have indicated the presence of a fitness cost associated with pyrethroid resistance in this species (Campanhola et al. 1991, Park 1998, Lee et al. 1999, Zhao et al. 2000). Published estimates of fitness-cost magnitudes, however, have until now been unavailable, presumably because they are difficult to obtain in a direct manner. We were able to obtain precise estimates of fitness-cost magnitudes relatively easily using the indirect method outlined in this study. Furthermore, relative fitness in this study was defined as the rate a genotype survives exposure to pyrethroids and lives to mate successfully. These types of relative fitness estimates are much more difficult to obtain using traditional direct methods, highlighting an additional benefit of the indirect method.

Unfortunately, parameter estimates obtained for the bollworm could not be compared with published estimates, except for degree of dominance. The degree of dominance implied by our estimates was much lower than dominance based on LD_{50} values for a population in South Carolina (Brown et al. 1998); however, the difference was not statistically significant. Instead, this result provides another example of how point estimates of dominance can differ depending on the method used to obtain them. In addition, this result indicates that more field research might be needed to improve confidence in dominance estimates for this species, especially in light of the fact that pyrethroids have been registered for use in field corn and grain sorghum in the midsouth (Ottea et al. 1998). As a result, a sound understanding of the nature of pyrethroid resistance may be important for designing efficient Bt and pyrethroid resistance management strategies for the midsouth.

Unrestricted and restricted relative fitness estimates under selection pressure were generally covariant and, as a result, statistically imprecise. This appears to be a drawback of the indirect estimation method. However, the restricted estimate of the relative fitness of bollworms homozygous for pyrethroid resistance was statistically different from zero, indicating that it is possible to obtain statistically precise relative fitness estimates when knowledge concerning other relative fitness parameters is incorporated in the estimation problem. In addition, migration and fitness costs associated with pyrethroid resistance that specifically affect winter survival were not incorporated in the simulation model. As a result, the presence of environmental effects on fitness, as well as the levels of parameters that may depend on actual migration rates and interseasonal fitness costs, should be interpreted with this in mind.

Acknowledgments

The authors would like to thank Ralph Bagwell, Fred Gould, George Kennedy, Roger Leonard, John Van Duyn, Jim Walgenbach, and two anonymous reviewers who provided comments and suggestions that led to significant improvements in the manuscript. Monsanto Company provided funds in partial support of this research.

References Cited

Abd-Elghafar, S. F., C. O. Knowles, and M. L. Wall. 1993. Pyrethroid resistance in two field strains of *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Econ. Entomol. 86: 1651–1655.

Abd-Elghafar, S. F., and C. O. Knowles. 1996. Pharmacokinetics of fenvalerate in laboratory and field strains of *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Econ. Entomol. 89: 590–593.

Bagwell, R., D. Cook, J. Adamczyk, B. Leonard, and S. Micinski. 2000. Status of insecticide resistance in budworm and bollworm in Louisiana during 1999, pp. 914–918. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Bourget, D., A. Genissel, and M. Raymond. 2000. Insecticide resistance and dominance levels. J. Econ. Entomol. 93: 1588–1595.

Burd, A., J. Bradley, Jr., J. Van Duyn, and F. Gould. 2000. Resistance of bollworm, *Helicoverpa zea*, to CryIA(c) toxin, pp. 923–926. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Burd, A., J. Bradley, Jr., J. Van Duyn, F. Gould, and W. Moar. 2001. Estimated frequency of non-recessive Bt resistance genes in bollworm, *Helicoverpa zea. In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Brown, T. M., P. K. Bryson, D. S. Brickle, S. Pimprale, F. Arnette, M. E. Roof, J. T. Walker, and M. J. Sullivan. 1998. Pyrethroid-resistant *Helicoverpa zea* and transgenic cotton in South Carolina. Crop Protect. 17: 441–445.

Campanhola, C., and F. W. Plapp, Jr. 1989. Toxicity and synergism of insecticides against susceptible and pyrethroid resistant strains third instars of the budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 82: 1495–1501.

Campanhola, C., B. F. McCutchen, E. H. Baehrecke, and F. W. Plapp, Jr. 1991. Biological constraints associated with resistance to pyrethroids in the budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 84: 1404–1411.

Caprio, M. 1998. Evaluating resistance management strategies for multiple toxins in the presence of external refuges. J. Econ. Entomol. 91: 1021–1031.

Caprio, M. A. and B. E. Tabashnik. 1992. Local adaptation and gene flow among finite populations: simulation of evolution of insecticide resistance. J. Econ. Entomol. 85: 611–620.

Carter, R. 1998. Bt cotton –the second year –a consultant's perspective, pp. 960–961. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Coleman, T., M. A. Branch, and A. Grace. 1999. Optimization toolbox for use with MATLAB. The MathWorks Inc., Natick, MA.

Croft, B. A. and J. Dunley. 1993. Habitat patterns and pesticide resistance, pp. 145–162. *In* H. C. Kim and B. A. McPheron (eds.), Evolution of insect pests. Wiley, New York.

Curtis, C. 1985. Theoretical models of the use of insecticide mixtures for the management of resistance. Bull. Entomol. Res. 75: 259–265.

Daly, J. C., and G. P. Fitt. 1990. Resistance frequencies in overwintering pupae and the first spring generation of *Helicoverpa armigera* (Lepidoptera: Noctuidae): selective mortality and immigration. J. Econ. Entomol. 83: 1682–1688.

Elzen, G. W., R. Leonard, J. Graves, E. Burris, and S. Micinski. 1992. Resistance to pyrethroid, carbamate, and organophosphate insecticides in field populations of budworm (Lepidoptera: Noctuidae) in 1990. J. Econ. Entomol. 85: 2064–2072.

Elzen, G. W., S. Martin, R. Leonard, and J. Graves. 1994. Inheritance, stability, and reversion of insecticide resistance in budworm (Lepidoptera: Noctuidae) field populations. J. Econ. Entomol. 87: 551–558.

Fletcher, R. 1987. Practical methods of optimization, 2nd ed. Wiley, New York.

Forrester, N. W., M. Cahill, L. J. Bird, and J. K. Layland. 1993. Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. Bull. Entomol. Res. Supple. 1 83: 1–132.

Gage, E. and L. Hatfield. 1989. Efficacy relationships of pyrethroid field use rates and vial test rates for *Heliothis virescens,* pp. 341–343. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Gallant, A. 1987. Nonlinear statistical models. Wiley, New York.

Georghiou, G. 1983. Management of resistance in arthropods, pp. 769–792. *In* G. Georghiou and T. Saito (eds.), Pest resistance to pesticides. Plenum, New York.

Gould, F. 1986. Simulation models for predicting durability of insect-resistance germ plasm: a deterministic diploid, two-locus model. Environ. Entomol. 15: 1–10.

Gould, F., A. Anderson, A. Jones, D. Sumerford, D. Heckel, J. Lopez, S. Micinski, R. Leonard, and M. Laster. 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens.* Proc. Natl. Acad. Sci. U.S.A. 94: 3519–3523.

Greene, W. 1993. Econometric analysis, 2nd ed. Macmillan, New York.

Kanga, L.H.B., F. W. Plapp, Jr., B. F. McCutchen, R. D. Bagwell, and J. D. Lopez, Jr. 1996. Tolerance to cypermethrin and endosulfan in field populations of the bollworm (Lepidoptera: Noctuidae) from Texas. J. Econ. Entomol. 89: 583–589.

Kennedy, G. G., and N. P. Storer. 2000. Life systems of polyphagous arthropod pests in temporally unstable cropping systems. Annu. Rev. Entomol. 45: 467–493.

Lambert, A., J. Bradley, Jr., F. Gould, and J. Van Duyn. 1998. Bollworm (*Helicoverpa zea*): adaptation to Bt toxin, pp. 1033–1037. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Layton, B., M. Williams, and S. Stewart. 1997. Bt-cotton in Mississippi: the first year, pp. 861–863. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Lee, D., Y. Park, T. M. Brown, and M. E. Adams. 1999. Altered properties of neuronal sodium channels associated with genetic resistance to pyrethroids. Mol. Parm. 55: 584–593.

Leonard, R., J. Graves, T. Sparks, and A. Pavloff. 1987. Susceptibility of bollworm and budworm larvae to pyrethroid and organophosphate insecticides, pp. 320–324. *In* Proceedings, Beltwide Cotton Production Research Conference, National Cotton Council, Memphis, TN.

Leonard, B., J. Graves, and A. Pavloff. 1988. Variation in resistance of field populations of budworm and bollworm (Lepidoptera: Noctuidae) to selected insecticides. J. Econ. Entomol. 81: 1521–1528.

Livingston, M. J. 1999. Insecticide resistance management policy. PhD dissertation, North Carolina State University, Raleigh, NC.

Luttrell, R. G. 1994. Cotton pest management. 2. A U.S. perspective. Annu. Rev. Entomol. 39: 527–542.

Mani, G. 1985. Evolution of resistance in the presence of two insecticides. Genetics 109: 761–783.

Mason, P. L. 1998. Selection for and against resistance to insecticides in the absence of insecticide: a case study of malathion resistance in the saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). Bull. Entomol. Res. 88: 177–188.

Muggleton, J. 1986. Selection for malathion resistance in *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae): fitness values of resistant and susceptible phenotypes and their inclusion in a general model describing the spread of resistance. Bull. Entomol. Res. 76: 469–480.

Nicholson, R., and T. Miller. 1985. Multifactorial resistance to trans-permethrin in field-collected strains of the budworm, *Heliothis virescens* F. Pestic. Sci. 16: 561–570.

Ottea, J. A., and J. W. Holloway. 1998. Target-site resistance to pyrethroids in *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie). Pestic. Biochem. Physiol. 61: 155–167.

Ottea, J. A., S. A. Ibrahim, A. M. Younis, B. R. Leonard, and A. R. McCaffery. 1995. Biochemical and physiological mechanisms of pyrethroid resistance in *Heliothis virescens* (F.). Pestic. Biochem. Physiol. 51: 117–128.

Park, Y. 1998. Evolutionary succession of pyrethroid resistance mutations in a sodium channel of *Heliothis virescens F.* Ph.D. dissertation, University of Arizona, Tucson.

Park, Y. and M.F.J. Taylor. 1997. A novel mutation L1029H in sodium channel gene *hscp* associated with pyrethroid resistance for *Heliothis virescens* (Lepidoptera: Noctuidae). Insect Biochem. Mol. Biol. 27: 9–13.

Park, Y., M.F.J. Taylor, and R. Feyereisen. 1997. A valine421 to methionine mutation in IS6 of the *hscp* voltage-gated sodium channel associated with pyrethroid resistance in *Heliothis virescens* F. Biochem. Biophysiol. Res. Comm. 239: 688–691.

Park, Y., D. Lee, M.F.J. Taylor, J. W. Holloway, J. A. Ottea, M. E. Adams, and R. Feyereisen. 2000. A mutation Leu1029 to His in *Heliothis virescens* F. hscp sodium channel gene associated with a nerve-insensitivity mechanism of resistance to pyrethroid insecticides. Pest. Biochem. Physiol. 66: 1–8.

Payne, G., R. Blenk, and T. Brown. 1988. Inheritance of permethrin resistance in the budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 81: 65–73.

Plapp, F., Jr., G. McWhorter, and W. Vance. 1987. Monitoring for pyrethroid resistance in the budworm in Texas–1986, pp. 324–326. *In* Proceedings, Beltwide Cotton Production Research Conference, National Cotton Council, Memphis, TN.

Plapp, F., Jr., C. Campanohola, R. Bagwell, and B. McCutchen. 1990a. Management of pyrethroid-resistant budworms on cotton in the United States, pp. 237–260. *In* R. Roush and B. Tabashnik (eds.), Pesticide resistance in arthropods. Chapman & Hall, New York.

Plapp, F., Jr., J. Jackman, C. Campanhola, R. Frisbie, J. Graves, R. Luttrell, W. Kitten, and M. Wall. 1990b. Monitoring and management of pyrethroid resistance in the budworm (Lepidoptera: Noctuidae) in Texas, Mississippi, Louisiana, Arkansas and Oklahoma. J. Econ. Entomol. 83: 335–341.

Rabb, R. L., and G. G. Kennedy (eds.). 1979. Movement of highly mobile insects: concepts and methodology in research. University Graphics, North Carolina State University, Raleigh, NC.

Roush, R. T. and R. G. Luttrell. 1987. The phenotypic expression of pyrethroid resistance in *Heliothis* and implications for resistance management, pp. 220–224. *In* Proceedings, Beltwide Cotton Production Research Conference, National Cotton Council, Memphis, TN.

Roush, R. T., and R. G. Luttrell. 1989. Expression of resistance to pyrethroid insecticides in adults and larvae of budworm (Lepidoptera: Noctuidae): Implications for resistance monitoring. J. Econ. Entomol. 82: 1305–1310.

Roush, R. T., and J. A. McKenzie. 1987. Ecological genetics of insecticide and acaracide resistance. Annu. Rev. Entomol. 32: 361–380.

Sims, S. 1995. Bacillus thuringiensis var. *kurstaki* [Cry1A(c)] protein expressed in transgenic cotton: effects on beneficial and non-target insects. Southwest Entomol. 20: 493–500.

Sorenson, C., A. Schreiber, H. Townsend, Jr., A. Abd-Elghafar, M. Fairchild, and C. Knowles. 1998. Monitoring pyrethroid resistance in bollworm (Lepidoptera: Noctuidae) moths in Missouri, 1988 to 1994. J. Entomol. Sci. 33: 300–312.

Sparks, T. 1981. Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America. Bull. Entomol. Soc. Am. 27: 186–192.

Staetz, C. 1985. Susceptibility of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) to permethrin from across the Cotton Belt: a five year study. J. Econ. Entomol. 78: 505–510.

Storer, N. P. 1999. The corn earworm, Bt transgenic corn and Bt-resistance evolution in a mixed cropping system. Ph.D. dissertation, North Carolina State University, Raleigh.

Suszkiw, J. 2001. Securing cotton farmers' Bt investment. News from the USDA Agricultural Research Service (http://www.ars.usda.gov/is/pr/2001/010212.htm).

Tabashnik, B. E., and B. Croft. 1985. Evolution of pesticide resistance in apple pests and their natural enemies. Entomophaga 30: 37–49.

Taylor, C. 1986. Genetics and evolution of resistance to insecticides. Biol. J. Linn. Soc. 27: 103–112.

Taylor, C., F. Quaglia, and G. Georghiou. 1983. Evolution of resistance to insecticides: a case study on the influence of migration and insecticide decay rates. J. Econ. Entomol. 76: 704–707.

Taylor, M.F.J., D. G. Heckel, T. M. Brown, M. E. Kreitman, and B. Black. 1993. Linkage of pyrethroid insecticide resistance to a sodium channel locus in the budworm. Insect Biochem. Mol. Biol. 23: 763–775.

Taylor, M.F.J., Y. Park, and Y. Shen. 1996. Molecular population genetics of sodium channel and juvenile hormone esterase markers in relation to pyrethroid resistance in *Heliothis virescens* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 89: 728–738.

Watson, T. and S. Kelly. 1991. Inheritance of resistance to permethrin by the budworm *Heliothis virescens* (F.): implications for resistance management. Southwest. Entomol. Suppl. 15: 135–141.

Williams, M. R. 1997. Cotton insect losses 1996, pp. 834–853. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Williams, M. R. 1998. Cotton insect loss estimates–1997, pp. 957–960. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Williams, M. R. 1999. Cotton insect losses 1998, pp. 785–806. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Williams, M. R. 2000. Cotton insect losses 1999, pp. 887–913. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Wolfenbarger, D., P. Bodegas, and R. Flores. 1981. Development of resistance in *Heliothis* spp. in the Americas, Australia, Africa, and Asia. Bull. Entomol. Soc. Am. 27: 181–185.

Zhao, Y., Y. Park, and M. Adams. 2000. Functional and evolutionary consequences of pyrethroid resistance mutations in S6 transmembrane segments of a voltage-gated sodium channel. Biochem. Biophysiol. Res. Comm. 278: 516–521.

Received for publication 14 June 2001; accepted 15 April 2002.